

REMARKS

The above amendments to the specification have been made to be in compliance with CFR §1.821-1.825. Accordingly, Applicant believes no new matter is added by these amendments.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

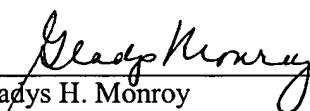
In the unlikely event that the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 220002059710.

The Assistant Commissioner is NOT authorized to charge the cost of the issue fee.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please replace the following paragraph starting on page 8 at line 16 and ending on page 10 at line 4

Accordingly, in certain embodiments, the invention provides mutant apo-B100 proteins and mutant apo-B100 polypeptide fragments, as well as LDL particles and other lipoproteins comprising a mutant apo-B100 protein or polypeptide fragment, which comprise a mutant Site B selected from one of the following Site B sequences:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:1);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:2);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ala**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:3);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Thr**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:4);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ser**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:5);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Gln**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:6);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Glu**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:7);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Asp**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:8);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:9);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:10);

Thr₃₃₅₈-**Glu**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:11);

and

Thr₃₃₅₈-**Asp**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:12);

as well as Site B sequences with deletions, such as:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-----Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:13);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-----Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:14);

and

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-----Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:15);

and Site B sequences which include insertions, such as:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:16);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:17);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:18); and

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:19).

Please replace the following paragraph starting on page 18 at line 14 and ending on line 17

The amino acid sequence of the wild-type human apo-B100 protein from amino acid 3358 to 3367 is as follows:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:20).

Please replace the following paragraph starting on page 19 at line 22 and ending on page 21 line 6

The following are the amino acid sequences from position 3358 to position 3367 for a list of preferred proteoglycan receptor⁺ apo-B100 protein mutants:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:1)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:2)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ala**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:3)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Thr**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:4)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ser**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:5)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Gln**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:6)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Glu**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:7)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Asp**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:8)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:9)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:10)

Thr₃₃₅₈-**Glu**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:11)

Thr₃₃₅₈-**Asp**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:12)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-----Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:13)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-----Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:14)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-----Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:15)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:16)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:17)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:18)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:19), wherein the repeated dashed lines represent deletions.

Please replace the following paragraph starting on page 37 at line 24 and ending on page 38 line 3

The polynucleotides of the invention can also be derivatized in various ways, including those appropriate for facilitating transfection and/or gene therapy. The polynucleotides can be derivatized by attaching a nuclear localization signal to it to improve targeted delivery to the nucleus. One well-characterized nuclear localization signal is the heptapeptide PKKKRKV (pro-lys-lys-lys-arg-lys-val) (SEQ ID NO:21). Preferably, in the case of polynucleotides in the form of a closed circle, the nuclear localization signal is attached via a modified loop nucleotide or spacer that forms a branching structure.

Please replace the following paragraph starting on page 39 at line 18 and ending on page 40 line 13

Moreover, Borén, J. et al. 1996. *Genome Res.* 6:1123-1130 have demonstrated how to isolate a 5.7 kb fragment of the apo-B100 gene which comprises Site B, in order to perform site-directed mutagenesis as described below in Examples 1 and 2, using RARE cleavage. In brief, RecA-assisted restriction endonuclease (RARE) cleavage consists of protecting a specific restriction endonuclease site with a complementary oligonucleotide. In the presence of RecA, a triplex DNA complex is formed that prevents methylation at the protected sites, for example *EcoRI*-35763 and *EcoRI*-41496 were protected by oligonucleotides (5' gaaaactccacagcaagctaattgattatctgaattcattcaattgggagagacaa gtttcac 3') (SEQ ID NO:22) and (5' cacaagtgaatatctggttaggatagaattctccagtttcacaatgaaaacatc 3') (SEQ ID NO:23) respectively, while unprotected sites are methylated by the corresponding methylase. After dissociation of the oligonucleotides, the protected sites can be cleaved with the restriction endonuclease which corresponds to the protected sites, for example *EcoRI*. All of the non-protected *EcoRI* site had been methylated and were thus not subject to cleavage by the restriction enzyme. The resulting fragment of the apo-B100 gene can then be ligated into a smaller vector which is appropriate for site-directed mutagenesis, e.g. pZER0. The site-directed mutagenesis process is then conducted by techniques well known in the art, and the fragment is return and ligated to the larger vector from which it was cleaved. For site directed mutagenesis methods see, for example, Kunkel, T. 1985. *Proc. Natl. Acad. Sci. U.S.A.* 82:488; Bandeyar, M. et al. 1988. *Gene* 65: 129-133; Nelson,

M., and M. McClelland 1992. *Methods Enzymol.* 216:279-303; Weiner, M. 1994. *Gene* 151: 119-123; Costa, G. and M. Weiner. 1994. *Nucleic Acids Res.* 22: 2423; Hu, G. 1993. *DNA and Cell Biology* 12:763-770; and Deng, W. and J. Nickoff. 1992. *Anal. Biochem.* 200:81.

Please replace the following paragraph starting on page 44 at line 25 and ending on page 45 line 9

The 95-kb apo-B P1 plasmid p158 (Linton, M. et al. 1993. *J. Clin. Invest.* 92:3029-3037) was prepared and modified by RARE cleavage as described by Borén, J. et al. 1996. *Genome Res.* 6:1123-1130. Oligomers *EcoRI*-35763 (5' gaaaactcccacagcaagctaattgattatctgaattcattcaattgggagagacaagtttcac 3') (SEQ ID NO:22) and *EcoRI*-41496 (5' cacaagtgaatatctggttaggatagaattctcccagtttcacaatgaaaacatc 3') (SEQ ID NO:23) were used to make 5.7-kb-deleted P1 plasmid. A 5.7-kb fragment was isolated from the apo-B100 "Leu-Leu" plasmid with RARE cleavage using oligomers *EcoRI*-35763 and *EcoRI*-41496 and cloned into the pZErO-1 vector (Invitrogen). The apo-B100 "Leu-Leu" plasmid was used to increase the yield of apo-B100, since it contains a CAA to CTA mutation in codon 2153 that effectively abolished the formation of apo-B48. The latter of which is formed by an editing mechanism present in mouse livers (Yao, Z. et al. 1992. *J. Biol Chem.* 267:1175-1182).

Please replace the following paragraph starting on page 45 at line 13 and ending on line 22

The pZErO-5.7 kb plasmid was subjected to site-directed mutagenesis with the Morph System (5 Prime→3 Prime, Inc.®) using oligonucleotide K3363E (5' caagattgacaagagaaaggggattgaag 3') (SEQ ID NO:24) to mutate the lysine at residue 3363 to glutamic acid. The resulting plasmids were subjected to RARE cleavage with oligomers *EcoRI*-35763 and *EcoRI*-41496, and the mutated 5.7-kb fragment was isolated. After RARE cleavage of the 5.7-kb-deleted P1 plasmid with oligonucleotide *EcoRI* del. 5.7-kb (5' ggaaaactcccacagcaagctaattgattatctgaattctccc agtttcacaatgaaaacatc 3') (SEQ ID NO:25), the mutated 5.7-kb fragment was ligated into the linearized and phosphatased 5.7-kb-deleted P1 vector (Borén, J. et al. 1996. *Genome Res.* 6:1123-1130).

In the Claims:

18. The apo-B100 protein according to claim 14, wherein said mutation in Site B is the K3363E mutation, and the amino acid sequence from position 3358 to 3359 is:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:1).